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Erythrocyte Insulin Binding in Preterm Newborn Infants

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ABSTRACT. To characterize the erythrocyte insulin receptor in newborn infants we studied the binding of ^{125}I-insulin to the erythrocytes from 42 preterm infants (14 at birth, 14 aged 2–7 days, and 14 aged 8–16 days) with a mean gestational age of 34.1 wk, and from 32 term infants (16 at birth and 16 aged 2–7 days). The insulin binding to cord blood erythrocytes from preterm infants was significantly higher than that of cord blood cells from term infants and to postnatal cells from preterm as well as term infants. The erythrocytes from preterm infants aged 2–7 days bound more insulin than cells from preterm infants aged 8–16 days. The maximum insulin binding (specific insulin binding at tracer concentration of insulin) correlated negatively with the gestational age both at birth and over the 1st postnatal wk. In the preterm infants there was a strong negative correlation between the maximum insulin binding and postnatal age. The enhanced insulin binding to cord blood erythrocytes from preterm infants was due to both an increased receptor concentration and a high affinity for insulin. The increased affinity persisted over the 1st wk of life. In preterm infants older than 1 wk the insulin binding characteristics were basically similar to those in term newborn infants. In all infants studied the receptor concentration seemed to be postnatal age dependent while the receptor affinity was gestational age dependent. No correlation was found between the insulin binding data and the plasma concentrations of immunoreactive insulin or C-peptide. The growth stimulatory effect of insulin in fetal life may be mediated by increased insulin binding to fetal cells, since preterm infants at birth have a high erythrocyte insulin binding capacity which decreases with increasing gestational age. The postnatal down-regulation of the erythrocyte insulin receptor in preterm infants may reflect the changing role of insulin from an intrauterine growth stimulating peptide to a hormone mainly regulating postnatal carbohydrate metabolism. (*Pediatr Res* 20: 256–260, 1986)

Abbreviations

IRI, immunoreactive insulin
K_e, empty site affinity
K_f, full site affinity

In general, alterations in the properties of human insulin receptor in normal conditions as well as during disease have been measured in receptor-bearing cells that are readily accessible, such as circulating monocytes and erythrocytes. The receptors on these cells generally reflect the status of receptors on the major target organs, although exceptions are known (1), and conclusions must be made with caution. In addition, discrepancies between insulin binding to monocytes and erythrocytes are known, for instance important differences have been shown in the way that their receptors are regulated (2, 3). The erythrocytes are the cells of choice for studies on insulin receptor in infants and small children, since small sample volumes are required. However, the effect of some physiological variables, such as age, cell age and gestational age on the erythrocyte insulin binding must be taken into account in these studies, otherwise the evaluations may result in misleading interpretations (4–7). We have previously demonstrated that insulin binding to erythrocytes is correlated to chronological age, being highest in cord blood erythrocytes (7). Cord blood erythrocytes from preterm infants have been found to bind even more insulin than those from term infants and, in addition, there is a strong negative correlation between the insulin binding to cord blood erythrocytes and gestational age (4, 6). The increased erythrocyte insulin binding in term infants at birth persists over the neonatal period and the major decrease in insulin binding occurs during the 1st yr of life (7). In the present study we have examined erythrocyte insulin binding in preterm infants over the first weeks of life in order to evaluate postnatal changes in the characteristics of their erythrocyte insulin receptor.

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MATERIALS AND METHODS

Subjects. We studied 14 preterm (group 1) and 16 term infants (group 4) at birth, 14 preterm (group 2) and 16 term infants (group 5) aged 2–7 days as well as 14 preterm infants (group 3) older than 1 wk. The clinical data of the subjects are presented in Table 1. The birth weight ratio was calculated as the ratio between the actual birth weight and the 50th percentile weight for that gestational age (8). Glucocorticoid treatment was given to two mothers in group 1, to one in group 2, and to two in group 3 prior to delivery. Ritadrine was infused to six mothers in group 1, to five in group 2, and to three in group 3 during premature labor. The neonatal course was complicated by a mild or moderate respiratory distress syndrome in two infants in group 1, in two in group 2, and in one in group 3. One baby in each group of preterm infants was treated with teophyllamine due to recurrent attacks of apnea. Hyperbilirubinemia necessitating phototherapy was seen in five infants in group 1, in three in groups 2, 3, and 4, respectively, and in five in group 5. One infant in group 1 and 2, respectively, was treated with exchange transfusion, which was, however, performed after the time of sampling. Neonatal hypoglycemia was not diagnosed in any of the infants studied. The data on the term infants have been published previously (7). Informed consent was obtained from the parents of the subjects. The gestational age of the infants was estimated from the menstrual history of the mother and from examination of the baby using the criteria of Dubowitz *et al.* (9). None of the mothers had any evidence of carbohydrate intolerance. A mixed arteriovenous cord blood sample was obtained at birth, whereas later a peripheral blood sample was taken after fasting for 4 hr.

Table 1. *Clinical data, mean ± SD (range), of the infants studied*

Group	*n*	Sex M/F	Age (days)	Gestational age (wk)	Apgar score 1 min	Apgar score 5 min	Birth wt (g)	Birth wt ratio	Postnatal wt loss Absolute (g)	Postnatal wt loss Relative (%)
1. Preterm infants	14	12/2	0	34.1 ± 1.6 (31–36)	7.2	8.2	2400 ± 534 (1720–3850)	1.05 ± 0.16	135 ± 107	5.3 ± 3.2
2. Preterm infants	14	9/5	4.5 ± 1.6 (2–7)	34.2 ± 1.3 (32–36)	8.2	8.1	2287 ± 409 (1560–2820)	1.01 ± 0.12	180 ± 64	7.0 ± 2.4
3. Preterm infants	14	5/9	11.3 ± 2.4 (8–16)	34.0 ± 1.4 (32–36)	8.2	8.8	2374 ± 507 (1500–3310)	1.1 ± 0.24	171 ± 101	7.6 ± 5.0
4. Term infants	16	8/8	0	39.6 ± 1.6 (37–42)	8.8	9.2	3631 ± 498 (2870–4350)	1.07 ± 0.13	213 ± 59	5.9 ± 1.5
5. Term infants	16	8/8	3.7 ± 0.9 (2–5)	39.2 ± 1.2 (37–42)	8.9	9.2	3469 ± 453 (2730–4230)	1.05 ± 0.12	203 ± 72	5.8 ± 1.8

Methods. The blood samples (10–15 ml) were collected into cooled heparinized tubes. Plasma was frozen for later analysis of IRI and C-peptide.

For the determination of mono-^{125}I (Tyr A14) insulin (0.25 ng/ml, specific activity 200–250 μCi/μg, Novo Research Institute, Bagsvaerd, Denmark) binding to erythrocytes the method of Gambhir *et al.* (10) was used with a few modifications (7). Specific insulin binding was defined as a total minus nonspecific binding, the latter determined by obtaining the values in the presence of 10^5 ng/ml unlabeled insulin. The amount of insulin specifically bound was corrected to a final erythrocyte concentration of 3.52×10^9 cells/ml. The nonspecific binding was about 10–16% of the total binding (1.9–2.5% of the total radioactivity). Binding data were analyzed by Scatchard plot and the average affinity profile was determined by the method of DeMeyts and Roth (11), in which the highest insulin concentration used for calculations was 100 ng/ml.

The blood glucose concentrations were measured by the hexokinase method (Boehringer Mannheim, Mannheim, FRG). Plasma IRI concentrations were measured radioimmunologically using antiserum M 8309 (Novo Research Institute) with a minor modification of the charcoal separation method described by Herbert *et al.* (12). Plasma C-peptide concentrations were determined according to Heding (13) using antiserum M 1230 (Novo Research Institute).

Statistical analyses. The statistical evaluation was performed using linear regression analysis and parametric one-way analysis of variance. A modified *t* test [Bonferroni (14)] was used for comparisons between two groups.

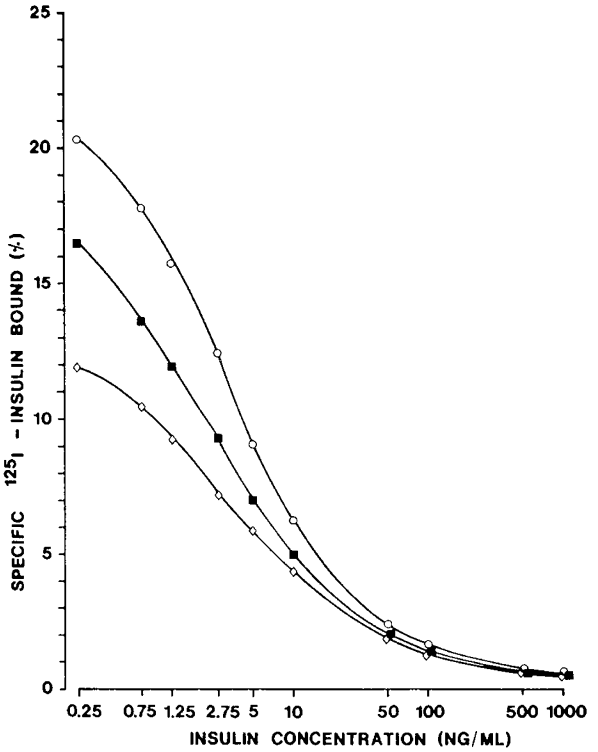

Fig. 1. The mean specific ^{125}I-insulin binding to 3.52×10^9 erythrocytes/ml in 14 cord blood samples from preterm infants (○), 14 preterm infants aged 2–7 days (■) and 14 preterm infants aged 8–16 days (◇).

RESULTS

The insulin binding to cord blood erythrocytes from preterm infants was significantly higher at tracer and physiological insulin concentrations ($p < 0.01$ or less) than was binding to cord blood cells from term infants or to postnatal cells from preterm as well as term infants (Fig. 1, Table 2). Although the insulin binding to the erythrocytes from preterm infants aged 2–7 days was not significantly higher than that from term infants of similar age ($p < 0.1$), it further decreased significantly ($p < 0.05$ or less) during the 2nd wk of life in preterm infants to a level that was slightly lower than that found in term infants aged 2–7 days. Ritadrine infusion to the mother during premature labor had no effect on the insulin binding at tracer concentration. The maximum insulin binding to cord blood erythrocytes as well as to those from newborn infants aged 2–7 days decreased with increasing gestational age (Fig. 2 *A* and *B*). In all preterm infants there was a strong negative correlation between maximum insulin binding and postnatal age (Fig. 3). In all subjects studied there was also a negative correlation between maximum insulin binding and gestational age ($r = -0.33$, $p < 0.01$) as well as the postnatal age ($r = -0.46$, $p < 0.001$). Furthermore, there was a positive correlation between the maximum insulin binding and the reticulocyte count ($r = 0.33$, $p < 0.01$) in all subjects studied, but no such correlation was found in the combined cord blood group or the combined group of newborn infants younger than 1 wk. The mean reticulocyte counts in cord blood erythrocytes (preterm: 2.3% and term: 2.1%) were significantly higher ($p < 0.01$ or less) than that found in postnatal erythrocytes (less than 1.1%). We found no correlation between the maximum insulin binding and the birth weight ratio or the postnatal weight loss. Neither was there any correlation between the maximum insulin binding and the contemporaneous blood glucose concentration or the lowest blood glucose level observed in the neonatal period.

The Scatchard analysis of the insulin binding data in the preterm infants is shown in Figure 4, and the number of binding sites per cell as well as the average affinity constants for empty and full sites in the preterm and term infants in Table 2. Compared with postnatal cells from preterm or term infants, cord blood erythrocytes from preterm infants had a significantly greater number of binding sites per cell ($p < 0.05$ or less). They

Table 2. *Maximum insulin binding, average affinity constants, and number of insulin receptors (mean ± SD) in five groups of infants*

Group	Maximum insulin binding (%)	Average affinity constants × 10^8 M^{-1}		No. of binding sites per cell
		K_e	K_f	
1. Preterm infants cord blood (n = 14)	20.27 ± 4.07	5.99 ± 1.97	1.01 ± 0.27	79.5 ± 12.6
2. Preterm infants aged 2–7 days (n = 14)	16.47 ± 2.91	6.15 ± 1.59	1.22 ± 0.30	60.3 ± 12.3
3. Preterm infants aged 8–16 days (n = 14)	11.95 ± 2.36	4.07 ± 1.23	1.02 ± 0.30	63.0 ± 16.9
4. Term infants cord blood (n = 16)*	14.03 ± 1.19	4.12 ± 0.85	0.86 ± 0.23	72.6 ± 12.4
5. Term infants aged 2–7 days (n = 16)*	13.85 ± 2.18	4.86 ± 1.26	0.79 ± 0.24	61.4 ± 14.3
Statistics				
$F_{4,69}$	20.45, $p < 0.001$	7.15, $p < 0.001$	5.74, $p < 0.001$	5.23, $p < 0.001$
Comparison between groups	1 *vs* 2 $p < 0.01$ 1 *vs* 3, 4, or 5 $p < 0.001$ 2 *vs* 3 $p < 0.001$	1 *vs* 3 or 4 $p < 0.01$ 2 *vs* 3 or 4 $p < 0.01$	2 *vs* 4 $p < 0.01$ 2 *vs* 5 $p < 0.001$	1 *vs* 2 or 5 $p < 0.01$ 1 *vs* 3 $p < 0.05$

* From Puukka *et al.* (7), used by permission of S. Karger AG, Basel.

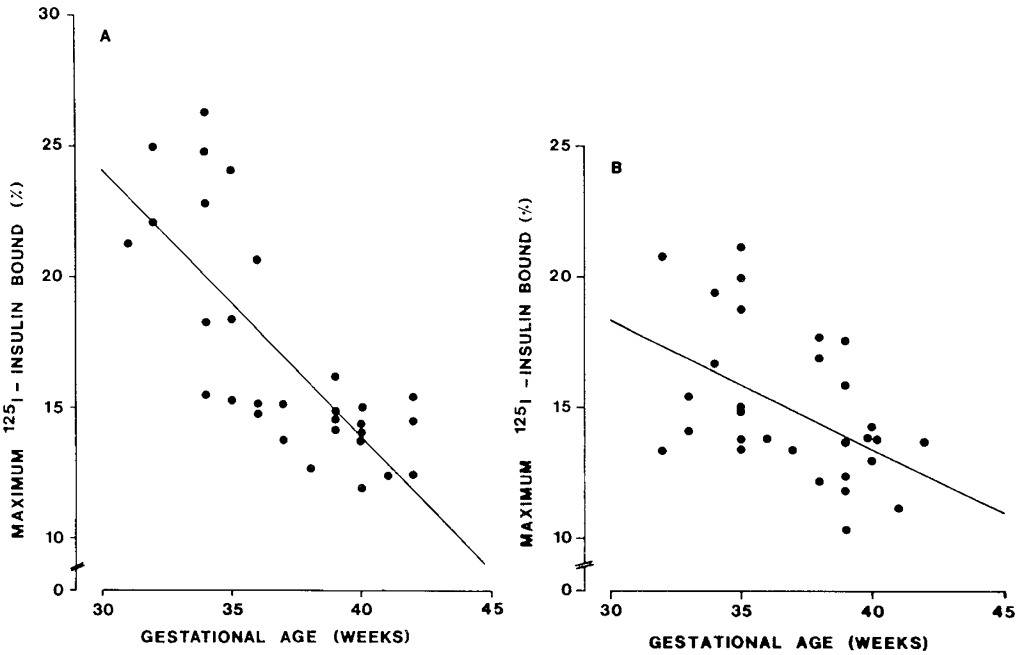

Fig. 2. Specific ^{125}I-insulin binding to erythrocytes at tracer concentrations in relation to gestational age. *A*, from 30 cord blood samples (y = −1.02x + 54.80, $r = -0.75$, $p < 0.001$) and *B*, from 30 infants aged 2–7 days (y = −0.49x + 33.02, $r = -0.50$, $p < 0.01$).

also had a significantly higher average affinity constant for K_e than the cord blood erythrocytes from term infants ($p < 0.01$). The increased receptor affinity for insulin persisted during the 1st wk of life in preterm infants, but decreased significantly ($p < 0.01$) during the 2nd postnatal wk to a level corresponding to that in the cord blood or postnatal cells from term infants. The erythrocytes from preterm infants aged 2–7 days also had a significantly higher average affinity constant for K_f than the cord blood cells or postnatal cells from term infants ($p < 0.01$ or less). K_e correlated negatively with the gestational age both at birth ($r = -0.59$, $p < 0.001$) and during the 1st wk of life ($r = -0.36$, $p < 0.05$). Similarly K_f correlated negatively with the gestational age (cord blood: $r = -0.32$, $p < 0.05$, and 1st wk: $r = -0.52$, $p < 0.01$). In all infants studied there was a negative correlation on one hand between the receptor concentration and postnatal age ($r = -0.23$, $p < 0.05$) and on the other hand between the affinity constants and gestational age (K_e: $r = -0.30$, $p < 0.01$; K_f: $r = -0.32$, $p < 0.01$).

The blood glucose concentrations and plasma levels of IRI and C-peptide of the infants were also measured (Table 3). There were no significant differences in the concentrations of blood glucose, plasma IRI, or C-peptide between the different groups

Table 3. *Blood glucose, plasma IRI, and C-peptide concentrations (mean ± SD) in five groups of infants*

Group	Blood glucose (mmol/liter)	IRI (mU/liter)	C-peptide (nmol/liter)
1. Preterm infants cord blood (n = 14)	4.0 ± 1.03	12.3 ± 14.60	0.21 ± 0.11
2. Preterm infants aged 2–7 days (n = 14)	3.6 ± 0.83	9.3 ± 6.39	0.26 ± 0.17
3. Preterm infants aged 8–16 days (n = 14)	4.0 ± 0.92	22.6 ± 28.94	0.34 ± 0.30
4. Term infants cord blood (n = 16)*	3.8 ± 0.49	12.4 ± 6.50	0.29 ± 0.12
5. Term infants aged 2–7 days (n = 16)*	3.8 ± 0.64	7.6 ± 3.68	0.25 ± 0.14
Statistics $F_{4,69}$	0.68, NS	2.24, NS	0.95, NS

* From Puukka *et al.* (7), used by permission of S. Karger AG, Basel.

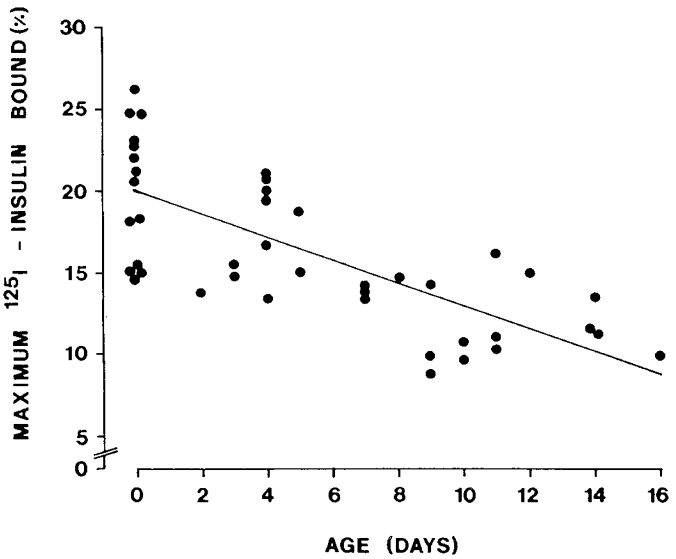

Fig. 3. Specific ^{125}I-insulin binding to erythrocytes at tracer concentrations in relation to postnatal age of preterm infants (y = −0.70x + 19.98, r = −0.72, $p < 0.001$).

of preterm and term infants and no significant correlations between the insulin binding data and the circulating concentrations of IRI or C-peptide.

DISCUSSION

The present results show that the considerably increased erythrocyte insulin binding in preterm infants at birth decreases over the first 2 wk of life to a level similar to that seen in term newborn infants. Previous studies have shown that cord blood erythrocytes from preterm infants have both an increased receptor concentration and an increased affinity for insulin when compared to erythrocytes from adults (6, 10), whereas cord blood cells from term infants are characterized by an increased receptor concentration only (7). Our observations indicate that the decrease in erythrocyte insulin binding seen in preterm newborn infants over the 1st wk of life is completely due to a substantial loss of binding sites, while the subsequent decline results from a reduced receptor affinity for insulin.

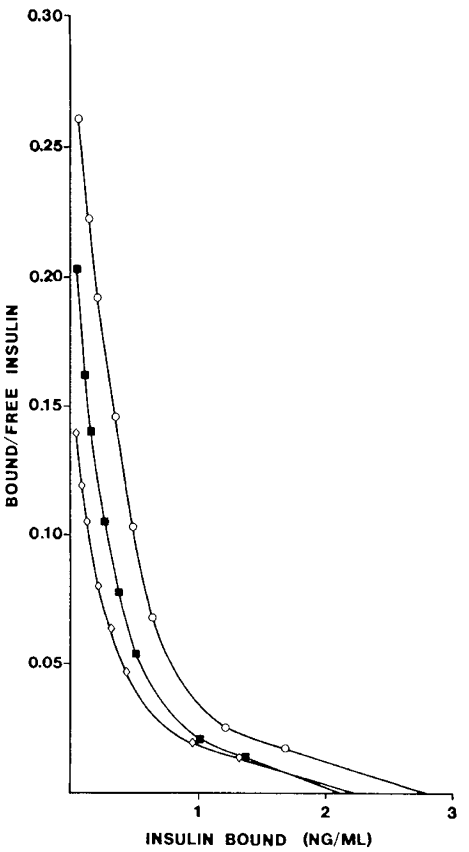

Fig. 4. Scatchard analysis of the data presented in Figure 1.

The drastic hematological changes which occur in the neonatal period (15) could be one explanation for the postnatal changes observed in the erythrocyte insulin receptors in preterm infants. The rate of production of red cells decreases dramatically during the first few days after birth, which diminishes the proportion of young cells and reticulocytes. This provides, at least partly, an explanation for the rapid postnatal decrease in receptor number observed both in preterm and term infant, since immature erythrocytes possess more receptor sites than do mature erythrocytes (5, 16). Although the life span of erythrocytes in preterm infants is very short, 30–50 days (15), the changes in the characteristics of the insulin receptors occur more rapidly than the destruction of fetal cells, indicating the existence of some regulation mechanism which responds to the altered function of insulin after birth. Obviously the erythrocytes from preterm infants modulate their insulin binding chiefly by alterations in their receptor affinity for insulin after birth, whereas the postnatal decrease in the receptor concentration is at least partly a consequence of hematological changes. The differences in receptor affinity must be due to factors other than cell maturity, since the presence of young cells does not affect the affinity (5). In erythrocytes from term infants most of the changes in the insulin receptor characteristics have already occurred prenatally.

In the fetus insulin is mainly needed for the regulation of growth (17), whereas the function of insulin alters after birth, when insulin also starts participating in the control of circulating blood glucose levels. Provided that the insulin binding to fetal erythrocytes mirrors binding characteristics of insulin receptors on actual target cells of insulin, the conspicuously high insulin binding to erythrocytes from preterm infants at birth emphasizes the crucial role of insulin in the regulation of fetal growth and offers a possible mechanism by which the growth stimulatory effect of insulin may be mediated. This hypothesis is supported by the decrease over the last trimester in insulin binding to fetal erythrocytes, as shown in the present study, and the concomitant decrease in fetal growth velocity, as previously observed (18). In

preterm infants without severe neonatal disease the postnatal growth proceeds more rapidly than in term newborn infants, but not as fast as in the intrauterine period (19). The changes in insulin binding occurring over the first weeks of life in preterm infants fit well with such a growth pattern. Preterm infants have an increased risk of developing hypoglycemia in the early neonatal period (20). The increased insulin binding in preterm infants at birth may contribute to the tendency to low blood glucose concentrations during the first few days of life, whereas a rapid postnatal decrease in insulin binding could protect the preterm infant from hypoglycemia later on.

Since the insulin binding to cord blood erythrocytes as well as to those from newborn infants decreased with increasing gestational age and was also correlated to postnatal age, it is essential to use controls matched for both gestational and chronological age in studies on erythrocyte insulin binding in the neonatal period. The receptor concentration seems to be age dependent, while the affinity for insulin seems to be gestational age dependent in newborn infants. As indicated previously (7), age-matched controls are also necessary throughout childhood.

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